JOURNAL OF BACTERIOLOGY, Apr. 1996, p. 2320–2327 Vol. 178, No. 8
0021-9193/96/$04.00+0

Isolation and Characterization of the *GFA1* Gene Encoding the Glutamine:Fructose-6-Phosphate Amidotransferase of *Candida albicans*

RACHEL J. SMITH,[1]* SŁAWOMIR MILEWSKI,[1,2] ALISTAIR J. P. BROWN,[1] AND GRAHAM W. GOODAY[1]

Molecular & Cell Biology, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, United Kingdom,[1] and Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 80-952 Gdansk, Poland[2]

Received 18 October 1995/Accepted 1 February 1996

Glutamine:fructose-6-phosphate amidotransferase (glucosamine-6-phosphate synthase) catalyzes the first step of the hexosamine pathway required for the biosynthesis of cell wall precursors. The *Candida albicans GFA1* gene was cloned by complementing a *gfa1* mutation of *Saccharomyces cerevisiae* (previously known as *gcn1-1*; W. L. Whelan and C. E. Ballou, J. Bacteriol. 124:1545–1557, 1975). *GFA1* encodes a predicted protein of 713 amino acids and is homologous to the corresponding gene from *S. cerevisiae* (72% identity at the nucleotide sequence level) as well as to the genes encoding glucosamine-6-phosphate synthases in bacteria and vertebrates. In cell extracts, the *C. albicans* enzyme was 4-fold more sensitive than the *S. cerevisiae* enzyme to UDP-*N*-acetylglucosamine (an inhibitor of the mammalian enzyme) and 2.5-fold more sensitive to N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (a glutamine analog and specific inhibitor of glucosamine-6-phosphate synthase). Cell extracts from the *S. cerevisiae gfa1* strain transformed with the *C. albicans GFA1* gene exhibited sensitivities to glucosamine-6-phosphate synthase inhibitors that were similar to those shown by the *C. albicans* enzyme. Southern hybridization indicated that a single *GFA1* locus exists in the *C. albicans* genome. Quantitative Northern (RNA) analysis showed that the expression of *GFA1* in *C. albicans* is regulated during growth: maximum mRNA levels were detected during early log phase. *GFA1* mRNA levels increased following induction of the yeast-to-hyphal-form transition, but this was a response to fresh medium rather than to the morphological change.

Candida albicans is an important pathogen in humans (34). Potentially significant virulence factors include its ability to undergo morphological transitions between yeast and hyphal growth forms and its efficiency at adhering to and invading host tissues (34, 35). Cells in the yeast form may circulate more freely in the bloodstream, whereas the mycelial cells appear to be better adapted for tissue invasion. The transition from the yeast to the hyphal form involves changes in synthesis of both chitin and mannoprotein (32, 52), both of which are essential components of the fungal cell wall and involved in host-fungus interactions. Indeed, hyphal cells express specific mannoproteins which are involved in selective binding to host components (for reviews, see references 8 and 9).

L-Glutamine:D-fructose-6-phosphate amidotransferase (glucosamine-6-phosphate synthase [Gfa1p or GFAT]; EC 2.6.1.16) catalyzes the formation of glucosamine-6-phosphate, i.e., the first step in the biosynthetic pathway leading to amino sugar-containing macromolecules such as glycoproteins and chitin (β1-4 homopolymer of *N*-acetylglucosamine). The eucaryotic glucosamine-6-phosphate synthase is subject to feedback inhibition by UDP-*N*-acetylglucosamine (UDP-GlcNAc), the substrate for chitin synthase in fungi, some protozoa, and most invertebrates (21). These two enzymes, glucosamine-6-phosphate synthase and chitin synthase, catalyze the first and last reactions, respectively, in the pathway required for the synthesis of chitin. The step catalyzed by glucosamine-6-phosphate synthase is essentially irreversible and, as such, is considered the committed step (19). In mammalian cells, glucosamine-6-phosphate synthase is an insulin-regulated enzyme which controls the flux of glucose into the hexosamine pathway. In the fungi *Blastocladiella emersonii* and *Aspergillus nidulans*, reversible phosphorylation-dephosphorylation appears to modulate sensitivity to feedback inhibition (6, 17). In *C. albicans*, chitin synthase activity is modulated both allosterically and by the rate of enzyme synthesis. The three chitin synthase genes are regulated at the transcriptional level during the dimorphic transition (11, 43).

Increased activity of the hexosamine pathway is important in fungi during morphological changes, such as during germ tube formation in *C. albicans*. This is accompanied by a three- to fivefold increase in the chitin content of the cell wall (10), a fourfold increase in the specific activity of glucosamine-6-phosphate synthase (12), and increased activity of *N*-acetylglucosamine kinase (44). A second example is the formation of the shmoo during the mating response of *Saccharomyces cerevisiae*. Here, among other changes, the addition of mating factors induces a fourfold increase in the levels of chitin in the cell wall (42) and the appearance of a specific O-glycosylated mannoprotein (36). This is reflected at the mRNA level: transcription of *GFA1* gene in **a** cells is stimulated by the addition of α factor (54).

Chitin/hexosamine biosynthesis is an important target for antimicrobial agents, and a number of antibiotics which affect the activity of glutamate synthetase are known. For example, tetaine (also known as bacilysin and bacillin) is a powerful inhibitor of *C. albicans* and particularly decreases the viability

* Corresponding author. Present address: Department of Biology, University of Michigan, Ann Arbor, MI 48109. Phone (313) 764-8500. Fax: (313) 747-0884.

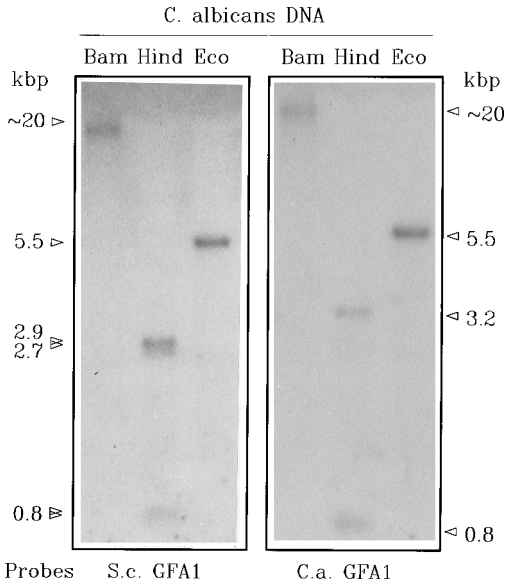

FIG. 1. Southern analysis of the *C. albicans GFA1* gene. *C. albicans* genomic DNA was digested with *Bam*HI, *Hin*dIII, or *Eco*RI and subjected to Southern blotting. Filters were probed with a 4-kb *Eco*RI fragment of YEpGW42 carrying the *S. cerevisiae GFA1* gene (S.c. GFA1 probe) (54), stripped, and then reprobed with *C. albicans GFA1* sequences (C.a. GFA1 probe) by using a mixed probe comprising two copurified 0.8-kb *Hin*dIII fragments from YCpCaGFA. The approximate lengths of the observed fragments are shown.

of the mycelial form (30). Tetaine is transported into the cells by a specific dipeptide permease and by the oligopeptide transport system in the yeast and hyphal forms, respectively. Once inside the cell, peptidases act to produce the C-terminal epoxy amino acid anticapsin. This acts as a glutamine analog and causes the irreversible inactivation of glucosamine-6-phosphate synthase in vitro (31). N^3-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) is another glutamine analog which acts as a specific inhibitor of glucosamine-6-phosphate synthase (28). Rationally designed FMDP-peptides show chemotherapeutic activity in the murine model of disseminated candidiasis (29). An increased understanding of the structure and mechanism of action of glucosamine-6-phosphate synthase from fungal pathogens would further increase its considerable potential as a target for antifungal drug therapy.

In this paper, we report the first isolation and characterization of a glucosamine-6-phosphate synthase gene from a fungal pathogen. This *C. albicans GFA1* gene is shown to complement a *gfa1* mutation in *S. cerevisiae* and to confer upon this mutant the profile of drug sensitivity normally associated with the *C. albicans* enzyme. We also report the existence of a single *GFA1* locus in *C. albicans*, the expression of which is regulated during growth and dimorphism.

MATERIALS AND METHODS

Chemicals. FMDP was synthesized by R. Andruskiewicz, Technical University of Gdansk.

Yeast strains and culture conditions. *C. albicans* Robin Berkhout 3153A was obtained from the London Mycological Reference Laboratory. *C. albicans* ATCC 10261 was a gift from M. Payton, Glaxo Institute for Molecular Biology, Geneva, Switzerland (45). These strains were grown to late exponential phase in the yeast form at 25°C with shaking at 200 rpm in YPD (2% glucose, 2% bacteriological peptone, 1% yeast extract). The yeast-to-hyphal-form transition was induced by transferring yeast cells to fresh medium containing 10% bovine calf serum at 37°C. *S. cerevisiae* XW270-2D (*MAT*α *gfa1-1 lys2-2*) was provided by C. E. Ballou (*gfa1* was previously known as *gcn1-1* [55]). XW270-2D was crossed with W303-1B (*MAT***a** *ade2 his3 leu2 trp1 ura3*) and sporulated to produce the strains RS417-1B (*trp1 ura3 his3 leu2 ade2 gfa1-1*) and RS418-8C (*trp1 ura3 gfa1-1*). *S. cerevisiae* BJ1991 (*MAT*α *pep4-3 prb1 ura3 leu2 trp1*) was provided by I. Purvis, Glaxo Group Research, Greenford, United Kingdom. *S. cerevisiae* was propagated in YPD with glucosamine added to 5 μg ml^{-1} when necessary or in defined medium containing 2% glucose, 0.65% yeast nitrogen base without amino acids, and the appropriate supplements at 50 μg ml^{-1}.

Bacterial strains, plasmids, and bacteriophages. *Escherichia coli* JM109 (57) and NM522 (22) were the host strains for M13 derivatives M13mp18 and M13mp19 (Life Technologies). DH5αF′ was used for plasmid selection and amplification. The *C. albicans* genomic library (provided by M. Payton) consisted of yeast DNA fragments of 9 to 13 kb obtained by partial *Sau*3A digestion of *C. albicans* ATCC 10261 DNA and insertion into the *Bam*HI site of YCp50 (45). The library was propagated in *E. coli* DH1. Plasmid YEpGW42 (provided by W. Tanner) carries the *S. cerevisiae GFA1* gene on a 4-kb *Eco*RI fragment inserted into YEp352 (54). Plasmid YCpCaGFA was isolated by complementation of the *gfa1-1* mutation in *S. cerevisiae* RS418-8C.

Preparation of soluble protein extract. *S. cerevisiae* or *C. albicans* strains were grown overnight at 25°C with vigorous shaking in YPD, harvested by centrifugation, and washed with 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA. Cells were disrupted in 50 mM potassium phosphate buffer (pH 6.5) containing 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol by using a small-scale glass bead disruption procedure (5). The broken-cell suspension was centrifuged at 27,000 × *g* for 30 min at 4°C. Cell extracts were prepared by passing the supernatant through a small Sephadex G-25 column which had previously been equilibrated with the disruption buffer.

Determination of glucosamine-6-phosphate synthase activity. A standard incubation mixture in a total volume of 1 ml consisted of 15 mM D-fructose-6-phosphate, 10 mM L-glutamine, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 50 mM potassium phosphate buffer (pH 6.5), and cell extract (final protein concentration, 0.15 to 1.5 mg ml^{-1}). The reaction was started by adding cell extract, incubated at 30°C for 30 min, and terminated by heating at 100°C for 1 min. The concentration of glucosamine-6-phosphate was determined by the modified Elson-Morgan procedure (24). Assays were performed in triplicate, and errors were less than 5%. One unit of specific activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of glucosamine-6-phosphate h^{-1} mg of $protein^{-1}$.

DNA isolation and manipulations. Yeast genomic DNA was prepared by the procedure of Boeke et al. (4). *S. cerevisiae* cells were transformed by the lithium

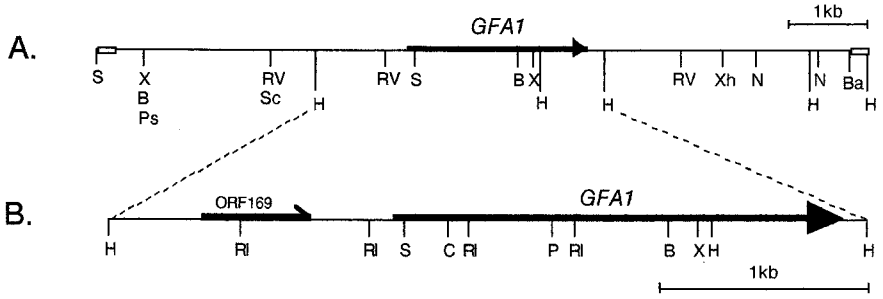

FIG. 2. Restriction map of the *C. albicans GFA1* locus. (A) Restriction map of the 9.5-kb fragment of *C. albicans* DNA from YCpCaGFA. The *GFA1* gene is shown as a heavy line. An upstream open reading frame is also shown (ORF169). Empty boxes indicate YCp50 DNA sequences flanking the *Bam*HI cloning site. Abbreviations for restriction endonucleases: B, *Bgl*II; Ba, *Bam*HI; C, *Cla*I; H, *Hin*dIII; N, *Nde*I; P, *Pvu*II; Ps, *Pst*I; RI, *Eco*RI; RV, *Eco*RV; S, *Sal*I; Sc, *Sac*I; X, *Xba*I; Xh, *Xho*I. Enzymes with no sites include *Sma*I, *Ksp*I, *Kpn*I, *Nru*I, *Nco*I, *Mlu*I, *Not*I, *Sph*I, *Apa*I, and *Aat*II. (B) Restriction map of 3.7 kb spanning the *GFA1* gene, as deduced from the DNA sequence. Only the sites used for subcloning are shown.

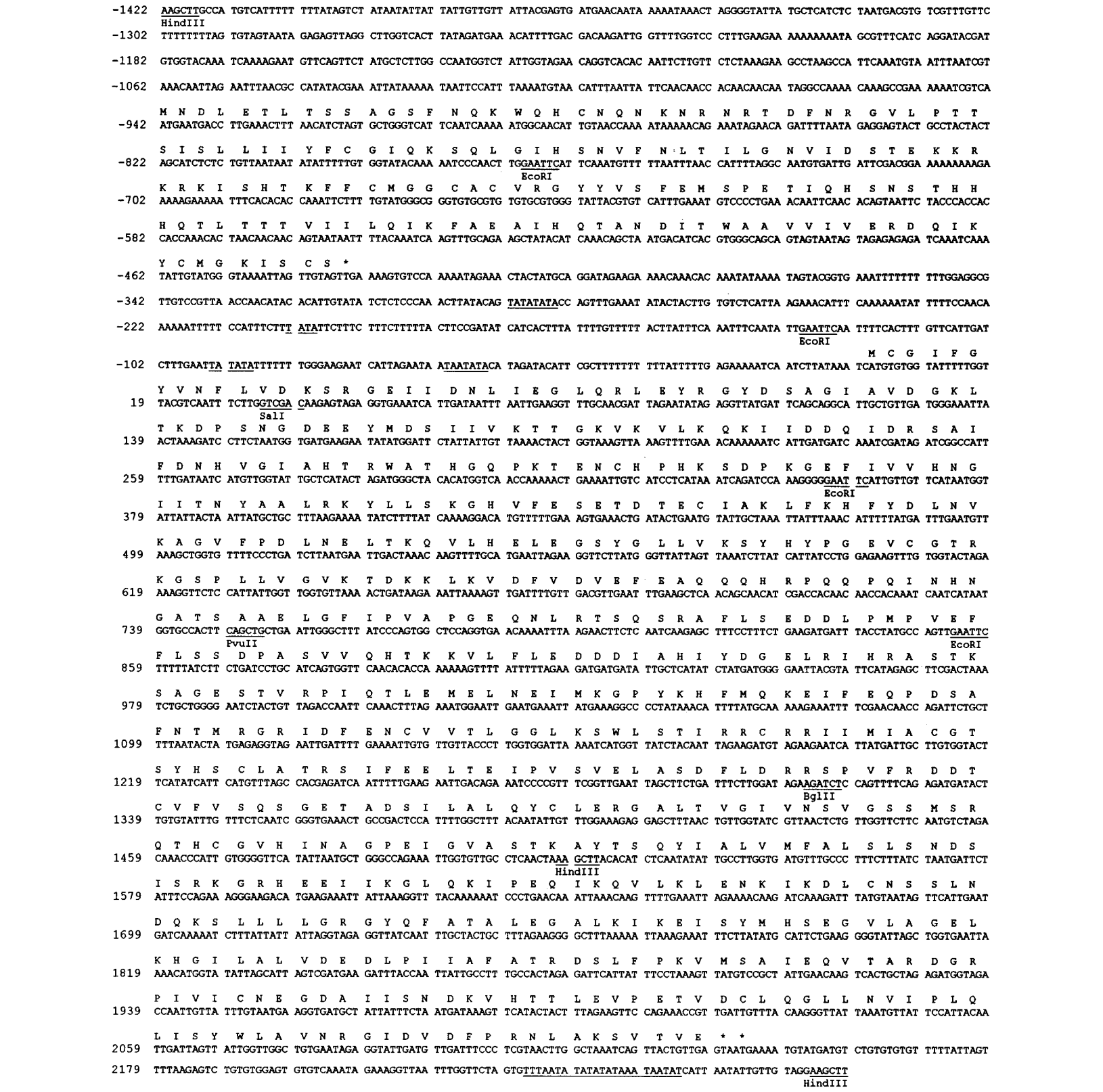

FIG. 3. Nucleic acid sequence of the *GFA1* locus. The DNA sequence presented covers 3,700 nucleotides. The open reading frame which corresponds to *GFA1* is shown, with the predicted amino acid sequence above. A second, upstream open reading frame corresponds to ORF169 (Fig. 2). Four potential TATA boxes are underlined. The A of the initial methionine codon is numbered 1. Two concurrent termination codons are marked with asterisks.

acetate method following the protocol of Ito et al. (23). Plasmids were recovered from *S. cerevisiae* by transformation of *E. coli* with a sample of lysed yeast extract (47). Molecular weight markers for electrophoresis were made by mixing lambda DNA digested with *Hin*dIII and with both *Hin*dIII and *Eco*RI. Standard procedures were used for the isolation and subcloning of plasmid DNA fragments (38).

DNA sequence analysis. The dideoxynucleotide chain termination methods of Sanger et al. (39) were used with Sequenase enzyme (United States Biochemical Corp.), [α-^{35}S]dATP, 1 μg of single-stranded M13 or 3 μg of denatured plasmid template, and the M13-40 sequencing primer or synthetic oligonucleotide primers. Segments of the *GFA1* gene were subcloned into M13mp18 and M13mp19 for sequencing on both strands. DNA sequences were analyzed with the Genetics Computer Group programs (15) on the SERC computer at the Daresbury Laboratory.

Southern analysis. Using procedures adapted from those of Southern (46), genomic DNA was digested to completion with a twofold excess of restriction enzyme, resolved by electrophoresis through a 0.8% agarose gel, denatured, and vacuum transferred (Vacugene; Pharmacia) to a nylon membrane (HiBond N; Amersham). Transfer buffer was 20× SSC (3 M NaCl, 0.3 M sodium citrate [pH 7.0]). The DNA was fixed by baking at 80°C for 2 h and prehybridized in a Hybaid bottle oven at 65°C in 5× SSC–0.5% sodium dodecyl sulfate (SDS)–5× Denhardt's solution (0.5% Ficoll, 0.5% bovine serum albumin, 0.5% polyvinylpyrrolidone)–200 μg of denatured salmon sperm DNA ml^{-1}. After 4 h, 5 ng of [α-^{32}P]dCTP-labelled restriction fragment was added (labelled at approximately 1 μCi ng of DNA^{-1} by primer extension [18]). Hybridizations were carried out for 20 h at 65°C, and then the filters were washed twice at 18°C for 2 min each time in 2× SSC–0.5% SDS and three times at 65°C for 15 min each time in 4× SSC–0.5% SDS (low stringency) or 0.1× SSC–0.5% SDS (high stringency).

RNA analysis. RNA was prepared by shearing the cells with glass beads and phenol-chloroform extraction, followed by two ethanol precipitation steps (7). After separation on a 1.2% agarose gel containing formaldehyde (38), the RNA was vacuum transferred to a HyBond-N membrane for 6 h. Hybridization was carried out in 40% formamide–5× Denhardt's solution–0.5% SDS–5× SSC–100 μg of denatured salmon sperm DNA ml^{-1}. After prehybridization for 4 h at 42°C, 25 ng of [α-^{32}P]dCTP-labelled probe was added and the filter was incubated overnight. Filters were washed twice for 5 min each in 2× SSC–0.5% SDS at 20°C and then three times for 15 min each in 0.5× SSC–0.5% SDS at 55°C. Signals were quantified directly by two-dimensional radioimaging with an AMBIS Radioanalytic System (LabLogic, Sheffield, United Kingdom) (33). To date, there has been no report of a *C. albicans* mRNA that remains at a sufficiently constant level for use as an internal loading control on Northern (RNA) blots (14, 49–51). Therefore, mRNA levels were measured relative to the rRNAs by loading approximately equal amounts (20 μg) of total RNA in each lane of the Northern gels (49–51).

Nucleotide sequence accession number. The EMBL accession number of the *GFA1* gene sequence is X94753.

RESULTS AND DISCUSSION

Isolation of the *C. albicans GFA1* gene. Before attempting to isolate the *C. albicans GFA1* gene, Southern analysis was performed to investigate the number of *C. albicans* loci with significant homology to the previously characterized *S. cerevisiae GFA1* gene (54). Genomic DNA from *C. albicans* 3153A was probed with the *GFA1* gene of *S. cerevisiae* (Fig. 1). When filters were hybridized under low-stringency conditions with the 4-kb *Eco*RI fragment from YEpGW42, single bands were observed in the *Eco*RI and *Bam*HI digests and three *Hin*dIII bands of 0.8, 2.7, and 2.9 kb were seen. This suggests that *C. albicans* has a single *GFA1* locus with at least two internal *Hin*dIII sites.

Since a number of *C. albicans* genes have been successfully identified by complementation of *S. cerevisiae* mutants (37), we isolated the *C. albicans GFA1* locus by complementing the glucosamine auxotrophy of *S. cerevisiae gfa1* mutants. In addition to this auxotrophy, which blocks essential chitin biosynthesis and protein N glycosylation (54), homozygous *gfa1* diploids fail to complete sporulation, forming spores with aberrant cell walls (55). The *S. cerevisiae gfa1* haploid, RS418-8C, was transformed with 5 μg of the YCp50 library (45), and about 4,000 Ura$^+$ transformants were screened on minimal medium lacking glucosamine. Plasmid YCpCaGFA was rescued by transforming *E. coli* DH5αF′ with a cell lysate from one *S. cerevisiae* transformant. Retransformation of YCpCaGFA into *S. cerevisiae* RS417-1B confirmed that this plasmid repaired the glucosamine auxotrophy displayed by a *gfa1-1* mutant.

Plasmid YCpCaGFA contained an insert of 9.5 kb. The restriction map of this insert (Fig. 2) was consistent with the hybridization patterns observed upon Southern blotting of *C. albicans* genomic DNA with the *S. cerevisiae GFA1* gene (Fig. 1). The *Hin*dIII digest of the cloned insert included two frag-

```
Ca   MCGIFGYVNFLVDKSRGEIIDNLIEGLQRLEYRGYDSAGIAVDGKLTKDPSNGDEEYMDS  60
Sc   MCGIFGYCNYLVERSRGEIIDTLVDGLQRLEYRGYDSTGIAIDG----------DEADST  50
Hu   MCGIFAYLNYHVPRTRREILETLIKGLQRLEYRGYDSAGVGFDGGNDKDW---EANACKT  57
Ec   MCGIVGAI------AQRDVAEILLEGLRRLEYRGYDSAGLAVV-----------DAEGHM  43
Rm   MCGIVGIV------GHQPVSERLVEALEPLEYRGYDSAGVATM-----------D-AGTL  42
Rl   MCGIVGIV------GHKPVSERLIEALGRLEYRGYDSSGVATI-----------F-EGEL  42
     **** .           .   . . *.  .*  ********.*..

Ca   IIVKTTGKVKVLKQKIIDDQ-IDRSAIFDNHVGIAHTRWATHGQPKTENCHPHKSDPKGE 119
Sc   FIYKQIGKVSALKEEITKQN-PNRDVTFVSHCGIAHTRWATHGRPEQVNCHPQRSDPEDQ 109
Hu   QLIKKKGKVKALDEEVHKQQDMDLDIEFDVHLGIAHTRWATHGEPSPVNSHPQRSDKNNE 117
Ec   TRLRRLGKVQMLAQAA-------EEHPLHGGTGIAHTRWATHGEPSEVNAHPHVSE---H  93
Rm   QRRRAEGKLGNLREKL-------KEAPLSGTIGIAHTRWATHGAPTERNAHPHFTE---G  92
Rl   HRRRAEGKLGNLKTRL-------KEAPLSGTVGIAHTRWATHGAPTECNAHPHFTD---G  92
        .  **.  *                .     *********** *    * **.  ..

Ca   FIVVHNGIITNYAALRKYLLSKGHVFESETDTECIAKLFKHFYDLNVKAGVFPDLNELTK 179
Sc   FVVVHNGIITNFRELKTLLINKGYKFESDTDTECIAKLYLHLYNTNLQNGHDLDFHELTK 169
Hu   FIVIHNGIITNYKDLKKFLESKGYDFESETDTETIAKLVKYMYDN--RESQDTSFTTLVE 175
Ec   IVVVHNGIIENHEPLREELKARGYTFVSETDTEVIAHLVNWE----LKQG--GTLREAVL 147
Rm   VAVVHNGIIENFAELKDELAAGGAEFQTETDTEVVAHLLAKY----RRDG--LGRREAMH 146
Rl   VAVVHNGIIENFSKLKDALAEVGTKFQTDTDTEVIAHLLTKF----RRDG--MGCLEAMH 146
       *.***** *    *.   *    *   *  ..**** .* *           . .

Ca   QVLHELEGSYGLLVKSYHYPGEVCGTRKGSPLLVGVKTDKKLKVDFVDVEFEAQQQHRPQ 239
Sc   LVLLELEGSYGLLCKSCHYPNEVIATRKGSPLLIGVKSEKKLKVDFVDVEFPEENAGQPE 229
Hu   RVIQQLEGAFALVFKSVHFPGQAVGTRRGSPLLIGVRSEHKLSTDHIPILYRT------- 228
Ec   RAIPQLRGAYGTVIMDSRHPDTLLAARSGSPLVIGLGMG--------------------- 186
Rm   AMLKRVKGAYALAVLFEDDPSTIMAARTGP-LAIGHGNG--------------------- 184
Rl   AMLKCVEGAFALAILFEDDPATIMVARNGPPLVIGHGDG--------------------- 185
       .  . *...          *      .* *. * .*

Ca   QPQINHN-----GATSAAELG-------FIPVAPGEQNLRTSQSRAFLSEDDLPMPVEFF 287
Sc   IPLKSNNKSFGLGPKKAREFEAGSQNANLLPIAANEFNLRHSQSRAFLSEDGSPTPVEFF 289
Hu   ----------GKDKKGSCNLSRVDSTTCLFPVEEK-------------------AVEYY 258
Ec   ---------------------------------------------------------ENF 189
Rm   ---------------------------------------------------------EMF 187
Rl   ---------------------------------------------------------EMF 188
                                                                  *

Ca   LSSDPASVVQHTKKVLFLEDDDIAHIYDGELRIHRASTKSAGESTVRPIQTLEMELNEIM 347
Sc   VSSDAASVVKHTKKVLFLEDDDLAHIYDGELHIHRSR-REVGASMTRSIQTLEMELAQIM 348
Hu   FASDASAVIEHTNRVIFLEDDDVAAVVDGRLSIHRIK-RTAGDHPGRAVQTLQMELQQIM 317
Ec   IASDQLALLPVTRRFIFLEEGDIAEITRRSVNIFDK----TGAEVKRQDIESNLQYDAGD 245
Rm   LGSDAIALAPFTNEITYLIDGDWAVIGKTGVHIFDF----DGNVVERPRQISTAAFLVD 243
Rl   LGSDAIALAPFTNDITYLNDGDWAVVGKTSVQVFDI----EGNVVTRPRHISLATADLVG 244
      .**  ..   *     .*  ..* * .     . .           *     *

Ca   KGPYKHFMQKEIFEQPDSAFNTMRGRIDFENCVVTLGGLKSWLSTIRRCRRIIMIACGTS 407
Sc   KGPYDHFMQKEIYEQPESTFNTMRGRIDYENNKVILGGLKAWLPVVRRARRLIMIACGTS 408
Hu   KGNFSSFMQKEIFEQPESVVNTMRGRVNFDDYTVNLGGLKDHIKEIQRCRRLILIACGTS 377
Ec   KGIYRHYMQKEIYEQPNAIKNTLTGRISHGQVDLSELG-PNADELLSKVEHIQILACGTS 304
Rm   KGNHRHFMEKEIYEQPEVIAIALGHYVNVIDKSCRSDS-DAID--FAGVESLAISCCGTA 300
Rl   KGNHPHFMEKEIYEQPEVIARALGHYINVNDSHVTTTS-TDID--FAGVESLAISACGTA 301
     **    .*.***.***.     ..    .    .     .            . .  ***.

Ca   YHSCLATRSIFEELTEIPVSVELASDFLDRRSPVFRDDTCVFVSQSGETADSILALQYCL 467
Sc   YHSCLATRAIFEELSDIPVSVELASDFLDRKCPVFRDDVCVFVSHCGETADTMLALNYCL 468
Hu   YHAGVATRQVLEELTELPVMVELASDFLDRNTPVFRDDVCFFLSQSGETADTLMGLRYCK 437
Ec   YNSGMVSRYWFESLAGIPCDVEIASEFRYRKSAVRRNSLMITLSQSGETADTLAGLRLSK 364
Rm   YLAGLIGKYWFERYARLPVEIAVASEFRYREIPLSPQSA-LFISQSGETADTLASLRYCK 359
Rl   YLAGLIGKYWFERYARLIVEIDVASEFRYREIPLSPRSAALFISQSGETADTLASLRYCK 361
     *  .  .   .*   .  .    . .**.*   *   ..         .*. *****.. .*

Ca   ERGALTVG-IVNSVGSSMSRQTHCGVHINAGPEIGVASTKAYTSQYIALVMFALSLSNDS 526
Sc   ERGALTVG-IVNSVGSSISRVTHCGVHINAGPEIGVASTKAYTSQYIALVMFALSLSDDR 527
Hu   ERGALTVG-ITNTVGSSISRETDCGVHINAGPEIGVASTKAYTSQFVSLVMFALMMCDDR 496
Ec   ELGYLGSLAICNVPGSSLVRESDLALMTNAGTEIGVASTKAFTTQLTVLLMLVANVSRLK 424
Rm   AHG-LRIGAVVNARESTMARESDAVFPILAGPEIGVARTKAFTCQLAVLAALRAGAGKAR 418
Rl   AHG-LRIGAVVNTRESTMAREADAIFPILAGPEIGVASTKAFTCQLAVLAALRIGAGKAR 420
        * *     . *    *.. *  ..        ** ***** ***.* *    *   .

Ca   ISRKGRHEE-IIKGLQKIPEQIKQVLKLENKIKDLCNSSLNDQKSLLLLGRGYQFATALE 585
Sc   VSKIDRIE--IIQGLKLIPGQIKQVLKLEPRIKKLCATELKDQKSLLLLGRGYQFAAALE 585
Hu   ISMQERRKE-IMLGLKRLPDLIKEVLSMDDEIQKL-ATELYHQKSVLIMGRGYHYATCLE 554
Ec   G-LDASIEHDIVHGLQALPSRIEQMLSQDKR-IEALAEDFSDKHHALFLGRGDQYPIALE 482
Rm   GTISGDEEQALIKSLAEMPAIMGQVLNSIQPEIEVLSRELSNCRDVLYLGRGTSFPLAME 478
Rl   GTITDDEEQVLVQSLATLPGVMRQVLNDITPEIELLSRELSHYRDVLYLGRGTSFPLAME 480
              .. .*   .*   . ..*                 . .     * .***   ..  .*

Ca   GALKIKEISYMHSEGVLAGELKHGILALVDEDLPIIAFATRDSLFPKVMSAIEQVTARDG 645
Sc   GALKIKEISYMHSEGVLAGELKHGVLALVDENLPIIAFGTRDSLFPKVVSSIEQVTARKG 645
Hu   GALKIKEITYMHSEGILAGELKHGPLALVDKLMPVIMIIMRDHTYAKCQNALQQVVARQG 614
Ec   GALKLKEISYIHAEAYAAGELKHGPLALIDADMPVIVVAPNNELLEKLKSNIEEVRARGG 542
Rm   GALKLKEISYIQPKSYAAGQLKHGPYALIDENMPVIVIAPHDRFFDKTVTNMQEVA-RGG 537
Rl   GALKLKEVSYIHAEGYAAGELKHGPIALIDENMPVIVIAPHDRFFDKTVSNMQEVAARGG 540
     ****.**..*...  .    **.****   **.*   .*.*        .     *     ...*  * *

Ca   RPIVICNEGDAIISNDKVHT---TLEVPETVDCLQGLLNVIPLQLISYWLAVNRGIDVDF 702
Sc   HPIIICNENDEVWAQKSKSIDLQTLEVPQTVDCLQGLINIIPLQLMSYWLAVNKGIDVDF 705
Hu   RPVVICDKEDTETIKNTKR----TIKVPHSVDCLQGILSVIPLQLLAFHLAVLRGYDVDF 670
Ec   QLYVFADQDAGFV---SS-DNMHIIEMPHVEEVIAPIFYTVPLQLLAYHVALIKGTDVDQ 598
Rm   RIILITDEKGAAA---SKLDTMHTIVLPEVDEIIAPMIFSLPLQLLAYHTAVFMGTDVDQ 594
Rl   RIILITDETGASM---SKLPTMHTIVLPDVAEIIAPMIFSLPLQLLAYHTAVVMGADVDQ 597
     .  .. .                        . .*.  .  .  ..  .****...  *.  * ***

Ca   PRNLAKSVTVE 713
Sc   PRNLAKSVTVE 716
Hu   PRNLAKSVTVE 681
Ec   PRNLAKSVTVE 609
Rm   PRNLAKSVTVE 605
Rl   PRNLAKSVTVE 608
     ***********
```

FIG. 4. Alignment of the predicted amino acid sequence of the *GFA1* open reading frame with glucosamine-6-phosphate synthase sequences from other species. The amino acid sequences of glucosamine-6-phosphate synthase from six organisms were aligned by using the CLUSTAL V program: *C. albicans* (Ca), *S. cerevisiae* (Sc), human (Hu), *E. coli* (Ec), *R. meliloti* (Rm), and *R. leguminosarum* (Rl). The numbers down the right-hand side are from the initial methionine residue for each sequence. Gaps introduced to maximize this alignment are shown by dashes, identical residues are indicated by asterisks, and similar residues are indicated by dots.

TABLE 1. Codon usage frequencies for *C. albicans GFA1*

Amino acid	Codon	Frequency	Amino acid	Codon	Frequency	Amino acid	Codon	Frequency	Amino acid	Codon	Frequency
F	TTT	19	S	TCT	27	Y	TAT	17	C	TGT	14
F	TTC	9	S	TCC	3	Y	TAC	2	C	TGC	0
L	TTA	45	S	TCA	15	*	TAA	1	*	TGA	0
L	TTG	19	S	TCG	4	*	TAG	0	W	TGG	3
L	CTT	5	P	CCT	9	H	CAT	22	R	CGT	2
L	CTC	0	P	CCC	2	H	CAC	1	R	CGC	0
L	CTA	0	P	CCA	16	Q	CAA	28	R	CGA	2
L	CTG[a]	0	P	CCG	0	Q	CAG	1	R	CGG	0
I	ATT	43	T	ACT	28	N	AAT	23	S	AGT	4
I	ATC	11	T	ACC	4	N	AAC	3	S	AGC	0
I	ATA	1	T	ACA	4	K	AAA	40	R	AGA	29
M	ATG	12	T	ACG	1	K	AAG	5	R	AGG	0
V	GTT	43	A	GCT	31	D	GAT	41	G	GGT	32
V	GTC	4	A	GCC	9	D	GAC	3	G	GGC	3
V	GTA	2	A	GCA	3	E	GAA	49	G	GGA	5
V	GTG	4	A	GCG	0	E	GAG	1	G	GGG	10

[a] The CTG codon is thought to encode serine in *C. albicans* (40, 41, 56).

ments of 0.8 kb, one of 2.7 kb, and one of 2.9 kb. The presence of *GFA1*-like sequences in YCpCaGFA was confirmed by Southern hybridization between this *C. albicans* genomic clone and the 4-kb *Eco*RI fragment from YEpGW42 carrying the *S. cerevisiae GFA1* gene (results not shown).

The two 0.8-kb *Hin*dIII fragments from YCpCaGFA (Fig. 2A) were copurified from an agarose gel and used to reprobe the Southern blot of *C. albicans* DNA. This yielded a 5.5-kb *Eco*RI fragment, a 20-kb *Bam*HI fragment, and a *Hin*dIII band of 0.8 kb (Fig. 1). Bands of similar lengths were observed with the *S. cerevisiae GFA1* probe, further indicating that the *C. albicans GFA1* locus had been cloned. An additional *Hin*dIII band of 3.2 kb was also observed on this Southern blot (Fig. 1). This band was due to the mixed probe, which, in addition to *GFA1* sequences, contained the 0.8-kb *Hin*dIII fragment from YCpCaGFA carrying the 3′ end of the genomic clone (Fig. 2).

Sequence analysis of *GFA1*. *GFA1* genes have been isolated previously from bacterial (2, 16, 48, 53), fungal (54), and mammalian (27) cells. The sequence of the *C. albicans GFA1* locus revealed an open reading frame of 2,139 bp, capable of encod-

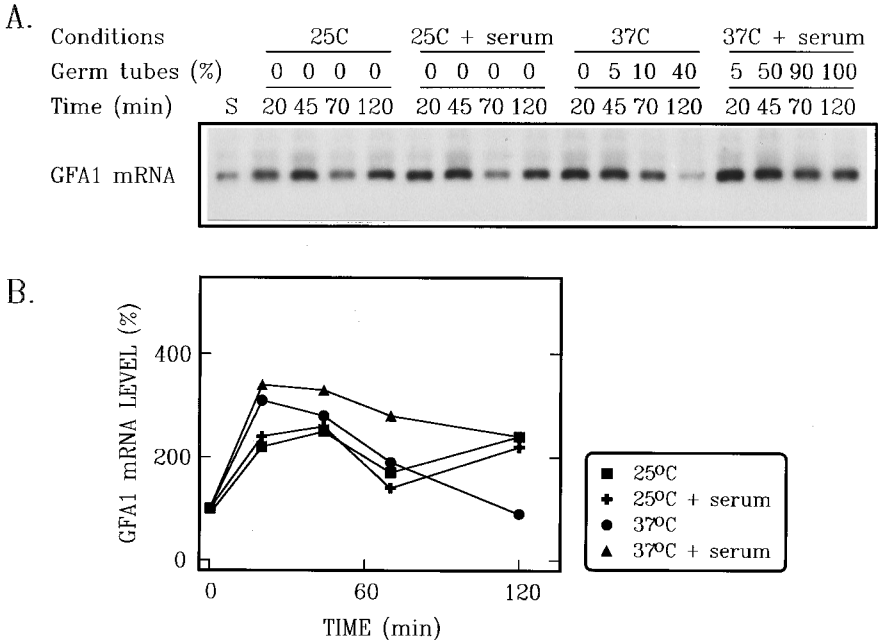

FIG. 5. *GFA1* mRNA levels during the yeast-to-hyphal-form transition. (A) An overnight starter culture of *C. albicans* 3153A (S) grown in YPD at 25°C was used to inoculate flasks containing either YPD at 25°C (25C), YPD containing serum at 25°C (25C + serum), YPD at 37°C (37C), or YPD containing serum at 37°C (37C + serum), and the proportion of cells forming germ tubes in each culture was determined by light microscopy. The levels of *GFA1* mRNA were measured at various times thereafter by quantitative Northern analysis. (B) The radioactive signals obtained on the filter shown in panel A were quantified directly by two-dimensional radioimaging. mRNA levels are presented relative to those in the starter culture immediately before dilution into fresh medium (100%).

ing a protein of 713 amino acids (Fig. 3) with homology to glucosamine-6-phosphate synthase from *S. cerevisiae* (84% similarity, 73% identity) (Fig. 4). Not surprisingly, the bacterial enzymes from *E. coli* and two *Rhizobium* species were less closely related to the *C. albicans* protein (59% similarity, 38 to 39% identity), although the two *Rhizobium* enzymes were very similar to each other (82% identity). The *C. albicans* protein was also homologous to the human (Fig. 4) and mouse (not shown) sequences. The mouse sequence is not included in the alignment, because it differed from the human sequence by only seven residues. These sequence comparisons suggest strongly that we had cloned the structural gene for the *C. albicans* glucosamine-6-phosphate synthase.

Glucosamine-6-phosphate synthase belongs to the group of *purF* amidotransferases (58). In the mature form, these enzymes contain a conserved N-terminal cysteine residue which functions in the glutamine amide transfer. This was also seen in the bacterial glucosamine-6-phosphate synthase (1). Such a residue is present in the predicted amino acid sequence of the *C. albicans* Gfa1 protein, assuming that the N-terminal methionine is removed in the course of posttranslational processing (Fig. 3). Lysine 708, present in the highly conserved C-terminal region of the *C. albicans* Gfa1 protein, probably corresponds to lysine 603 in the *E. coli* glucosamine-6-phosphate synthase (numbering without the N-terminal methionine). This residue is involved in the binding of D-fructose-6-phosphate (20).

Comparison of eukaryotic (*C. albicans*, *S. cerevisiae*, and human) and prokaryotic (*E. coli*, *Rhizobium leguminosarum*, and *R. meliloti*) glucosamine-6-phosphate synthase sequences reveals a relatively large region (residues 219 to 284 according to the *C. albicans* numbering) that is lacking in the prokaryotic proteins. Since eukaryotic glucosamine-6-phosphate synthases differ from the bacterial enzymes in that the former are subject to allosteric inhibition by UDP-GlcNAc (see Table 2) (25), it seems possible that this region contains amino acids involved in the interaction with the allosteric effector.

The G+C content of the *GFA1* sequence was consistent with that observed previously for *C. albicans* sequences (35% G+C): 32.8% for the structural gene and 29.9% for the upstream sequences. The codon usage frequencies (Table 1) reflected a low bias and were consistent with a *C. albicans* gene that is not highly expressed (26). The *GFA1* sequence contains no CTG codons which are translated as serine instead of leucine in *C. albicans* (40, 41, 56).

A 1.42-kb segment of flanking DNA upstream from the *GFA1* gene was also sequenced. Four TATA-like sequences were found at −55, −88, −197, and −285 with respect to the translation initiation codon. The context of the proposed initiation codon (AAAUCAUGUGU) was not dissimilar to that proposed by Cigan and Donahue (13) to promote favorable translation initiation in *S. cerevisiae* ([A/Y]A[A/U]AAUGUCU).

A second open reading frame, which encoded a possible 169-amino-acid polypeptide, was identified. It started at position −942 upstream from the *GFA1* coding sequence and continued to position −420 (Fig. 3). This open reading frame showed no significant similarity to any sequence in the GenBank-EMBL databases, and therefore its function remains obscure. There are two TATA-like sequences at −84 and −95 relative to the start of this open reading frame, and between −16 and −47 there are two stretches of CAA repeats. Regions of CAA repeats have been identified in the leader regions of other *C. albicans* genes, but their functional significance is not yet known (3).

***GFA1* gene regulation.** Previous reports indicate that the activity of the hexosamine pathway rises during germ tube formation in *C. albicans* (12, 44). Hence, we measured *GFA1* mRNA levels during the yeast-to-hyphal-form transition. Hyphal growth was induced by transferring *C. albicans* 3153A yeast cells grown in YPD at 25°C to fresh medium containing 10% bovine calf serum at 37°C, and RNA was isolated at various times up to 120 min. RNA was also analyzed from control cultures grown in fresh YPD at 25°C without serum, 25°C with serum, or 37°C without serum (Fig. 5). The *GFA1* probe detected a single band on the Northern blots corresponding to an mRNA of about 2.5 kb and consistent with the *GFA1* open reading frame of 2,139 bp (Fig. 3). Like other mRNAs involved in chitin biosynthesis (43), the *GFA1* mRNA was present at low levels (approximately 200-fold lower than the abundant alcohol dehydrogenase mRNA [50]).

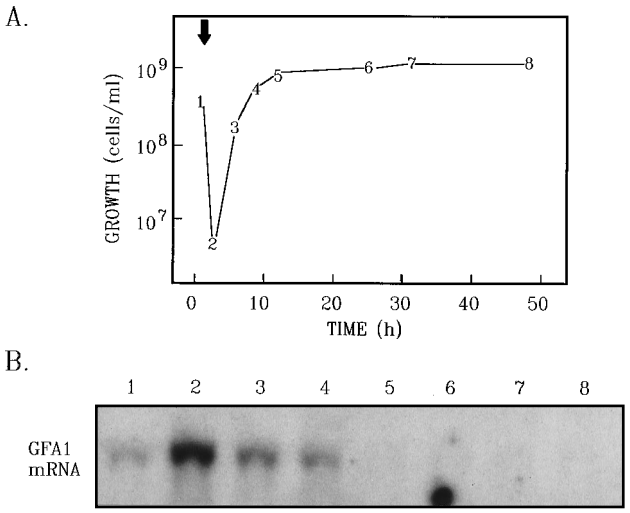

FIG. 6. *GFA1* mRNA levels during growth of the yeast form in YPD at 25°C. (A) An overnight starter culture of *C. albicans* 3153A was used to inoculate a fresh culture of YPD (arrow). The culture was incubated at 25°C with shaking (200 rpm), cell numbers were monitored by light microscopy, and cells were harvested for Northern analysis at the points shown (samples 2 to 8). (B) Northern analysis was performed on 20 μg of RNA prepared from the starter culture (sample 1) and from cells harvested at various times during growth (samples 2 to 8). Radioactive signals in most samples were too low to be quantified by two-dimensional radioimaging (<10 cpm).

The *GFA1* mRNA level increased about threefold when hyphal development was stimulated, but similar increases were observed in the control cultures (Fig. 5), suggesting that *GFA1* expression was responding to the fresh medium rather than morphogenesis. To test whether *GFA1* expression was regulated during growth, Northern analysis was performed on RNA prepared at various times during the growth of *C. albicans* 3153A in the yeast form on YPD at 25°C (Fig. 6). The level of the *GFA1* mRNA was at its highest in early exponential growth phase, becoming low in late exponential phase and undetectable in stationary phase. Hence, *GFA1* appears to respond to growth and the concomitant need for cell wall biosynthesis rather than to morphogenesis. This is consistent with previous reports which indicate that numerous *C. albicans* genes are influenced by the underlying physiological changes that accompany morphogenesis rather than the change in cell shape per se. These include glycolytic, actin, translation elongation factor 3, and ribosomal protein 10 mRNAs (14, 49–51).

Glucosamine-6-phosphate synthase activity in *S. cerevisiae* transformants. Glucosamine-6-phosphate synthase activities in cell extracts from *S. cerevisiae* BJ1991 (*GFA1*), RS417-1B (*gfa1-1*), and RS417-1B transformed with YCpCaGFA and

TABLE 2. Glucosamine-6-phosphate synthase activity in cell extracts and IC_{50}s for inhibitors

Strain	Glucosamine synthase sp act[a] (μmol h^{-1} mg of $protein^{-1}$)	IC_{50} of:	
		UDP-GlcNAc (mM)	FMDP[c] (μM)
S. cerevisiae BJ1991 (*GFA1*)	0.20	2.50	10.0
C. albicans 10261 (*GFA1*)	0.28	0.67	4.0
S. cerevisiae RS417-1B (*gfa1-1*)	0.0	NA[b]	NA
S. cerevisiae RS417-1B × YCpCaGFA	0.36	0.62	3.9

[a] Amidotransferase specific activities were measured in triplicate in cell extracts. Errors were less than 5%.
[b] NA, not applicable.

from *C. albicans* ATCC 10261 were measured (Table 2). Enzyme activity was not detected in RS417-1B. However, when this *gfa1* strain was transformed with YCpCaGFA, glucosamine-6-phosphate synthase levels comparable to those for BJ1991 were measured. This further confirmed the isolation of the structural *GFA1* gene from *C. albicans.*

The extent of inhibition of the *S. cerevisiae* and *C. albicans* glucosamine-6-phosphate synthases by UDP-GlcNAc and FMDP was also studied (Table 2). The concentration of UDP-GlcNAc required to give 50% inhibition (IC_{50}) was 2.5 mM for the *S. cerevisiae* enzyme and 0.62 to 0.67 mM for the *C. albicans* enzyme. FMDP, a glutamine analog and specific inhibitor of glucosamine-6-phosphate synthase, gave IC_{50}s of 10 μM for the *S. cerevisiae* enzyme and 4.0 μM for the *C. albicans* enzyme. Hence, the *C. albicans* enzyme was more sensitive to both inhibitors. The IC_{50}s for the extracts from the *S. cerevisiae gfa1* cells transformed with the *C. albicans GFA1* gene were similar to those obtained with *C. albicans* ATCC 10261, confirming the presence of the *C. albicans GFA1* gene product in this transformant and suggesting that the activity of the enzyme was not affected by possible differences in posttranslational modifications between these yeasts. Therefore, overexpression of the *C. albicans GFA1* gene in *S. cerevisiae* would appear to provide a useful route toward the analysis of the structure and function of the *C. albicans* enzyme, which represents a potential target for antifungal drugs.

ACKNOWLEDGMENTS

We thank C. Ballou for *S. cerevisiae* XW270-2D, W. Tanner for plasmid YEpGW42, and M. Payton (Glaxo Institute for Molecular Biology, Geneva, Switzerland) for the *C. albicans* genomic library. We also thank Rolf Swoboda and Gwyneth Bertram for RNA samples used for Northern analysis. We are grateful to Bernie Hube, Mick Tuite, Neil Gow, Barton Wicksteed, Rolf Swoboda, and Gwyneth Bertram for advice and discussion.

This work was supported by the Science and Engineering Research Council and The Wellcome Trust. S.M. was a Royal Society Postdoctoral Fellow. A.J.P.B. was awarded an Aberdeen University Research Fellowship.

REFERENCES

1. **Badet, B., P. Vermoote, P. Y. Haumont, F. Lederer, and F. LeGoffic.** 1987. Glucosamine synthetase from *Escherichia coli*: purification, properties and glutamine-utilizing site location. Biochemistry **26:**1940–1948.
2. **Baev, N., G. Endre, G. Petrovics, Z. Banfalvi, and A. Kondorosi.** 1991. Six nodulation genes of *nod* box locus 4 in *Rhizobium meliloti* are involved in nodulation signal production: *nodM* codes for D-glucosamine-6-phosphate synthetase. Mol. Gen. Genet. **228:**113–124.
3. **Bertram, G., R. K. Swoboda, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown.** 1996. Structure and regulation of the *Candida albicans* gene encoding an immunogenic alcohol dehydrogenase. Yeast **12:**115–127.
4. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40:**491–500.
5. **Bollag, D. M., and S. J. Edelstein.** 1991. Protein methods. Wiley-Liss Inc., New York.
6. **Borgia, P. T.** 1992. Roles of the *orlA*, *tsE* and *bimG* genes of *Aspergillus nidulans* in chitin synthesis. J. Bacteriol. **174:**384–389.
7. **Brown, A. J. P.** 1994. RNA extraction and mRNA quantitation in *Candida albicans*, p. 127–134. *In* B. Marasca and G. S. Kobayashi (ed.), Molecular biology of pathogenic fungi: a laboratory manual. Telos Press, New York.
8. **Calderone, R. A.** 1993. Recognition between *Candida albicans* and host cells. Trends Microbiol. **1:**55–58.
9. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. **55:**1–20.
10. **Chattaway, F. W., M. R. Holmes, and A. J. E. Barlow.** 1968. Cell wall composition of the mycelial and blastospore forms of *Candida albicans*. J. Gen. Microbiol. **51:**367–376.
11. **Chen-Wu, J. L., J. Zwicker, A. R. Bowen, and P. W. Robbins.** 1992. Expression of chitin synthase genes during yeast and hyphal growth phases of *Candida albicans*. Mol. Microbiol. **6:**497–502.
12. **Chiew, Y. Y., M. G. Shepherd, and P. A. Sullivan.** 1980. Regulation of chitin synthesis during germ-tube formation in *Candida albicans*. Arch. Microbiol. **125:**97–104.
13. **Cigan, A. M., and T. F. Donahue.** 1987. Sequence and structural features associated with translational initiation regions in yeast—a review. Gene **59:** 1–18.
14. **Delbrück, S., and J. F. Ernst.** 1993. Morphogenesis-independent regulation of actin transcript levels in the pathogenic yeast *Candida albicans*. Mol. Microbiol. **10:**859–866.
15. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
16. **Dutka-Malen, S., P. Mazodier, and B. Badet.** 1988. Molecular cloning and overexpression of the glucosamine synthetase gene from *Escherichia coli*. Biochimie **70:**287–290.
17. **Etchebehere, L. C., and J. C. C. Maia.** 1989. Phosphorylation-dependent regulation of amidotransferase during the development of *Blastocladiella emersonii*. Arch. Biochem. Biophys. **272:**301–310.
18. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
19. **Ghosh, S., H. J. Blumenthal, E. Davidson, and S. Roseman.** 1960. Enzymatic synthesis of glucosamine-6-phosphate. J. Biol. Chem. **235:**1265–1273.
20. **Golinelli-Pimpaneau, B., and B. Badet.** 1991. Possible involvement of Lys-603 from *Escherichia coli* glucosamine-6-phosphate synthase in the binding of its substrate fructose-6-phosphate. Eur. J. Biochem. **201:**175–182.
21. **Gooday, G. W.** 1990. Chitin metabolism as a target for antifungal and antiparasitic drugs. Pharmacol. Ther. **1990S:**175–185.
22. **Gough, J. A., and N. E. Murray.** 1983. Sequence diversity among related genes for recognition of specific targets in DNA molecules. J. Mol. Biol. **166:** 1–19.
23. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells with alkali cations. J. Bacteriol. **153:**163–168.
24. **Kenig, M., E. Vandamme, and E. P. Abraham.** 1976. The mode of action of bacilysin and anticapsin and biochemical properties of bacilysin-resistant mutants. J. Gen. Microbiol. **94:**46–54.
25. **Kornfeld, R.** 1967. Studies on 1-glutamine D-fructose-6-phosphate amidotransferase. J. Biol. Chem. **242:**3135–3141.
26. **Lloyd, A. T., and P. M. Sharp.** 1992. Evolution of codon usage patterns: the extent and nature of divergence between *Candida albicans* and *Saccharomyces cerevisiae*. Nucleic Acids Res. **20:**5289–5295.
27. **McKnight, G. L., S. L. Mudri, S. L. Mathews, R. R. Traxinger, S. Marshall, P. O. Sheppard, and P. J. O'Hara.** 1992. Molecular cloning, cDNA sequence, and bacterial expression of human glutamine:fructose-6-phosphate amidotransferase. J. Biol. Chem. **267:**25208–25212.
28. **Milewski, S., H. Chmara, R. Andruszkiewicz, and E. Borowski.** 1985. Synthetic derivatives of N^3-fumaroyl-L-2,3-diaminopropanoic acid inactivate glutamine synthetase from *Candida albicans*. Biochim. Biophys. Acta **828:**247–254.
29. **Milewski, S., H. Chmara, R. Andruszkiewicz, E. Borowski, M. Zaremba, and J. Borowski.** 1988. Antifungal peptides with novel specific inhibitors of glucosamine-6-phosphate synthase. Drugs Exp. Clin. Res. **14:**461–465.
30. **Milewski, S., H. Chmara, and E. Borowski.** 1983. Growth inhibitory effect of antibiotic tetaine on yeast and mycelial forms of *Candida albicans*. Arch. Microbiol. **135:**130–136.
31. **Milewski, S., H. Chmara, and E. Borowski.** 1986. Antibiotic tetaine—a selective inhibitor of chitin and mannoprotein biosynthesis in *Candida albicans*. Arch. Microbiol. **145:**234–240.
32. **Molinari, A., M. J. Gomez, P. Crateri, A. Torosantucci, A. Cassone, and G. Arancia.** 1993. Differential cell surface expression of mannoprotein epitopes in yeast and mycelial forms of *Candida albicans*. Eur. J. Cell Biol. **60:** 146–153.
33. **Moore, P. A., F. A. Sagliocco, R. M. C. Wood, and A. J. P. Brown.** 1991. Yeast glycolytic mRNAs are differentially regulated. Mol. Cell. Biol. **11:**5330–5337.
34. **Odds, F. C.** 1988. Candida and candidosis: a review and bibliography, 2nd ed. Bailliere Tindall, London.

35. **Odds, F. C.** 1994. *Candida* species and virulence. ASM News **60:**313–318.
36. **Orlean, P., H. Ammer, M. Watzele, and W. Tanner.** 1986. Synthesis of an O-glycosylated cell surface protein induced in yeast by α-factor. Proc. Natl. Acad. Sci. USA **83:**6263–6266.
37. **Rosenbluh, A., M. Mevarech, Y. Koltin, and J. A. Gorman.** 1985. Isolation of genes from *Candida albicans* by complementation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **200:**500–502.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
40. **Santos, M. A. S., G. Keith, and M. F. Tuite.** 1993. Non-standard translational events in *Candida albicans* mediated by an unusual seryl-tRNA with a 5′-CAG-3′ (leucine) anticodon. EMBO J. **12:**607–616.
41. **Santos, M. A. S., and M. F. Tuite.** 1995. The CUG codon is decoded in vivo as serine and not leucine in *Candida albicans*. Nucleic Acids Res. **23:**1481–1486.
42. **Schekman, R., and V. Brawley.** 1979. Localized deposition of chitin on the yeast cell surface in response to mating pheromone. Proc. Natl. Acad. Sci. USA **76:**645–649.
43. **Schofield, D. A., B. Hube, G. W. Gooday, and N. A. R. Gow.** Unpublished data.
44. **Shepherd, M. G., H. M. Ghazali, and P. A. Sullivan.** 1980. *N*-acetyl-D-glucosamine kinase and germ-tube formation in *Candida albicans*. Exp. Mycol. **4:**147–159.
45. **Smith, D. J., M. Cooper, M. DeTiani, C. Losberger, and M. A. Payton.** 1992. The *Candida albicans PPM1* gene encoding phosphomannomutase complements a *Saccharomyces cerevisiae sec53-6* mutation. Curr. Genet. **22:**501–503.
46. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
47. **Strathern, J. N., and D. R. Higgins.** 1991. Recovery of plasmids from yeast into *Escherichia coli*: shuttle vectors. Methods Enzymol. **194:**319–329.
48. **Surin, B. P., and J. A. Downie.** 1988. Characterization of the *Rhizobium leguminosarum* genes *modLMN* involved in efficient host-specific nodulation. Mol. Microbiol. **2:**173–183.
49. **Swoboda, R. K., G. Bertram, D. R. Colthurst, M. F. Tuite, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown.** 1994. Regulation of the gene encoding translation elongation factor 3 during growth and morphogenesis in *Candida albicans*. Microbiology **140:**2611–2616.
50. **Swoboda, R. K., G. Bertram, S. Delbrück, J. F. Ernst, N. A. R. Gow, G. W. Gooday, and A. J. P. Brown.** 1994. Fluctuations in glycolytic mRNA levels during morphogenesis in *Candida albicans* reflect underlying changes in growth and not a response to cellular dimorphism. Mol. Microbiol. **13:**663–672.
51. **Swoboda, R. K., I. D. Broadbent, G. Bertram, S. Budge, G. W. Gooday, N. A. R. Gow, and A. J. P. Brown.** 1995. Structure and regulation of a *Candida albicans RP10* gene which encodes an immunogenic protein homologous to *Saccharomyces cerevisiae* ribosomal protein 10. J. Bacteriol. **177:**1239–1246.
52. **Torosantucci, A., M. Boccanera, I. Casalinuovo, G. Pellegrini, and A. Cassone.** 1990. Differences in the antigenic expression of immunomodulatory mannoprotein constituents on yeast and mycelial forms of *Candida albicans*. J. Gen. Microbiol. **136:**1421–1428.
53. **Walker, J. E., N. J. Gay, M. Saraste, and A. N. Eberle.** 1984. DNA sequence around the *Escherichia coli unc* operon. Biochem. J. **224:**799–815.
54. **Watzele, G., and W. Tanner.** 1989. Cloning of the glutamine:fructose-6-phosphate amidotransferase gene from yeast. J. Biol. Chem. **264:**8753–8758.
55. **Whelan, W. L., and C. E. Ballou.** 1975. Sporulation in D-glucosamine auxotrophs of *Saccharomyces cerevisiae*: meiosis with defective ascospore wall formation. J. Bacteriol. **124:**1545–1557.
56. **White, T. C., L. E. Andrews, D. Maltby, and N. Agabian.** 1995. The "universal" leucine codon CTG in the secreted aspartyl proteinase 1 (*SAP1*) gene of *Candida albicans* encodes a serine in vivo. J. Bacteriol. **177:**2953–2955.
57. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
58. **Zalkin, H.** 1993. The amidotransferases. Adv. Enzymol. **66:**203–309.